Monitoring of pathogens of carrot (*Dausis Carota*) rot during storage in the territory of the Russian Federation

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Abstract. A study was conducted to determine the spectrum of pathogens that cause carrot rot when stored in storage conditions on 70 samples from 20 regions of the Russian Federation. Wet chamber method, microscopy, culture and molecular (PCR analysis) methods were used to identify pathogens. The most often dominant pathogens on carrot roots in storage conditions in all regions were fungi of the genus *Penicillium, Fusarium, Mucor, Aspergillus, Pythium, Alternaria; Botrytis cinerea, Sclerotinia sclerotiorum* и *Sclerotinia nivalis.* As a result of a study of the effectiveness of LLC "Syngenta" chemical plant protection products against white rot *in vitro* Miravis®, SC (adepidyn 200 g/l) и Cidely Top, SL (diphenoconazole 125 g/l + cyflufenamide 15 g/l), it was concluded that the treatment based on adepidine has a biological efficacy equal to 100%, compared with the other - with a biological efficacy of 33.3%.

1 Introduction

Carrots are an important part of the human diet. According to the recommendations of the Ministry of Health of Russia, this culture is among the recommended ones for diets and vitamin deficiency in the body [1]. "Damage by various microorganisms occurs at all stages of growth and development of the culture, therefore, timely detection of the first signs of the disease, as well as their correct diagnosis, is important" [2]. Otherwise, the development of diseases leads to a decrease in yields, the presentation of products and their quality are violated. Carrot losses in storage conditions can reach up to 70% [3]. If the technology of cultivation and harvesting is not followed, mechanical damage occurs on the surface of root

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crops. These lesions become a gateway to infection [4]. The most common cause of crop losses is non–compliance with storage conditions, in which the crop weakens,
loses its natural resistance to rot pathogens, which allows them to actively develop [5-6]. However, not all farmers can afford expensive machinery and equipment for non-traumatic harvesting of root crops and maintaining proper storage conditions for products [7]. The best way to control root rot's pathogens is genetic modification of plants. This way is the most safety for health then using treatment during the storage [8]. Therefore, the effectiveness of the use of chemical plant protection products during the growing season and its effect on the yield of products after storage is being studied.

The purpose of this study is to monitor the pathogens of carrot rot in various regions of the Russian Federation under storage conditions and to study the effectiveness of a number of chemical remedies against white rot.

2 Materials and methods of research

The work took place in the diagnostic laboratory of LLC "Syngenta" in 2022-2023. The object of the study was carrot root crops from various regions of the Russian Federation: the Republic of Tatarstan, Altai, Stavropol and Krasnoyarsk Territories, Astrakhan, Bryansk, Volgograd, Nizhny Novgorod, Kurgan, Moscow, Nizhny Novgorod, Novgorod, Omsk, Penza, Samara, Sverdlovsk, Tver, Tula, Tyumen, Yaroslavl regions.

The wet chamber method was used to isolate pathogens. The wet chamber method was used to isolate pathogens. To do this, the affected fragments of root crops with the capture of healthy tissue were placed in sterile petri dishes with filter paper moistened with sterile water. The wet chambers were placed in a thermostat with a temperature of 22 °C. After 3 days in a humid chamber, the obtained sporulation was identified by microscopy. If it was impossible to determine the pathogen, a cultural method was used. The obtained mycelium was sown on potato-glucose agar (PGA). If it was necessary to isolate the fungus into pure culture, antibiotics were added to the nutrient medium: streptomycin 0.05 grams dissolved in 3 ml of distilled sterile water and chloramphenicol 0.25 grams in 2 ml of ethyl alcohol. Antibiotics were prepared per 1 liter of PGA.

In the absence of conidia, the mycelium isolated in a humid chamber or on a agar was identified by classical PCR and Sanger sequencing. The amplification program for classical PCR consisted of the following stages: 95°C - 5 min; 95°C - 15 sec; 60°C - 10 sec; 72°C - 40 sec (35 cycles); 72°C - 5 min [9].

Identification of white rots is morphologically impossible, as fungi of the genus *Sclerotinia* do not have a conidial stage. For this purpose, specific primers were developed in the laboratory of Syntol LLC to enable pathogen identification using real-time PCR: "... based on sequences from the NCBI GenBank database, a pair of primers Scler_ Seq_F (5'-TGCTGCTTTCTGGTACGAAATC-3') and Scler_Seq_R (5'-ATACTTGTTGCCGGAAGCCTTG-3') was developed, allowing for species identification of *S. sclerotiorum*, *S. minor*., *S. nivalis* on the gene encoding β-tubulin (tub)" [2, 9].

The use of molecular diagnostics, such as species-specific primers, is crucial for the accurate identification of white rot fungi infecting carrots. This information can then be applied to breeding programs aimed at developing carrot varieties with enhanced resistance to storage diseases. Understanding the unique features of the causal agents will enable the selection of cultivars that can withstand prolonged storage periods while preserving their marketable attributes.

DNA was extracted from the mycelium of the isolated pure pathogen culture or from infected carrot tissues using the "Fitosorb-P" reagent kit with magnetic particles from Sintol LLC for manual or automated nucleic acid isolation. Samples were amplified on a CFX96 Touch Real-Time PCR Detection System using specific primers.

A positive result was considered to be 27-35 cycles, indicating the presence of the target pathogen DNA sequence. This approach enables the rapid and accurate identification of white rot fungi infecting carrot crops, which is crucial for the development of disease-resistant cultivars through breeding programs [9, 12].

The evaluation of the efficacy of preparations was carried out by the method of infecting carrot cross-sections and carrot leaves with plugs of the pathogen culture. For this purpose, the analyzed plant parts, previously washed under running water and sterilized with ethyl alcohol, were placed in containers with moist filter paper. After the evaporation of alcohol, the carrots and leaves were treated with a sprayer with solutions of the preparations. The volume of the solution per 1 container was 500 µl. After drying, a 2 mm plug of pure *Sclerotinia sclerotiorum* culture obtained from a carrot sample from the Samara region was placed on the surface of the sections and leaves, and the containers were loosely covered with lids. The paper was moistened daily.

After the experiment was set up, the containers were placed in a thermostat without lighting at a temperature of 24 degrees. The first accounting was carried out on the 3rd day after infection.

To assess the damage to carrot plants, a scoring scale from 0 to 4 points was formed, where: "0" - complete absence of signs of damage; "1" - abundant juice secretion at the inoculation sites, the diameter of the lesion was up to 4 mm in diameter, no mycelium was formed; "2" - the diameter of the lesion from 5 to 10 mm. In the necrosis site, the tissues are wet, easily depressed, and their integrity is disturbed, no mycelium is formed; "3" - the diameter of the lesion more than 10 mm, the tissues are macerated and darkened, mycelium appears on the surface; "4" - 90% of the surface of the section or carrot leaf is affected, the formation of sclerotia is possible.

The effectiveness of plant protection products they were tested in a wet chamber on carrot leaves and fragments using solutions of Miravis ®, SC (adepidine 200 g/l) in concentrations 0.18%, 0.25%, 0.30%, 0.33% and Cideli Top ™, SL (diphenoconazole 125 g/l + cyflufenamide 15 g/l) with a concentration of 0.19%, 0.25%, 0.30%, 0.33%. The development of the disease and the biological effectiveness of the pesticides were determined according to generally accepted formulas [10]. Assessment of the degree of damage to root crops was carried out by a visual method using an assessment scale 3 and 6 days after the lesion.

3 Results

A spectrum of pathogenic fungi was isolated from the obtained carrot samples of different varieties (Figure 1).

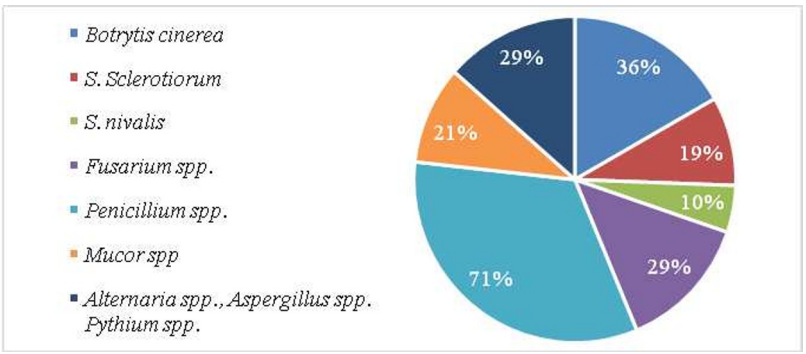

Fig. 1. The ratio of pathogenic micromycetes of rot of carrot in the storage period.

The most common pathogens (71%) were fungi of the genus *Penicillium*, which cause penicillous, or blue, rot of root crops. This disease frequently manifested itself under wet conditions in the form of isolated sporulation pads. The second most common (36%) was the causative agent of gray rot *Botrytis cinerea*. This pathogen appears on plants as an olive-grey sporulation composed of branching hyphae with elliptical spores after three days in a moist environment. Upon receiving the samples, carrots were covered in a grey, cottony mycelium on which sclerotia had formed. In almost 30% of the examined root crops, fungi of the genus *Alternaria* were identified. Pathogens of this genus often affect the aboveground organs of plants. *Fusarium spp.* affects the roots of the plant, penetrating through wounds and mechanical damage, causing rotting and the formation of white mycelium. Most often, these pathogens were found on root crops with a large amount of soil sticking to them. Along with these micromycetes, there were samples on which mold fungi caused rot *Mucor spp., Aspergillus spp*. These pathogens were manifested on depleted, flaccid carrot roots. As a result of their activity, all products were covered with the mycelium and fruiting bodies of the pathogens. Such products were of poor quality and unfit for human consumption. In some regions, Pythium spp. is also common. Fungi from this genus cause wet rot, which leads to the softening of the root crops and the release of abundant moisture.

Fungus *Penicillium spp., Mucor spp., Fusarium spp., Aspergillus spp.,* they are widespread almost everywhere and were found in many samples, they do not carry such economic significance. Most of the presented list are preserved on plant residues and soil lumps on root crops after harvesting, which leads to their minor further damage during storage [2].

Pathogens of white rot are preserved in plant residues in the form of sclerotia, which germinate with mycelium in the storage and reinfect carrots with fragments of mycelium, including those remaining on the walls of containers in which carrots are stored. Fungi, pathogens of white rot - *Sclerotinia sclerotiorum* and *Sclerotinia nivalis* are less common. But they are the most harmful than other pathogenic microorganisms of carrot [5, 11]. These are necrotrophic fungi that quickly infect plants, first softening the tissues with the help of special enzymes. Losses from damage by these pathogens can be more than 70%.

The white rot lesion is characterized by maceration of the root tissue. In severe cases, the tissue becomes soft and mushy, and can easily be damaged when touched. Subsequently, a thick and dense mycelial mat develops on the surface of the affected area. As the plant tissue continues to decompose, the mycelium thickens, and large sclerotia may form on its surface.

In establishing the spectrum of pathogens causing rots, a visual comparison was made of three difficult-to-identify pathogens: *Sclerotinia sclerotiorum* and *Sclerotinia nivalis*, which do not form a conidial stage, and *Botrytis cinerea*, which under certain conditions develops in a similar way to white rots. On carrots, when obtaining samples from the regions, fungi of the genus *Sclerotinia* formed a dense white mycelium, which was a single whole. Sclerotia were large, up to 2 cm in length, black on the outside, and white on the cut. However, on PGA, representatives of the genus develop differently. *Sclerotinia sclerotiorum* forms large sclerotia up to 1.5 cm, of irregular shape, formed along the edges of the colony. When the mycelium or sclerotium is placed in the center of the Petri dish, the sclerotia will form at the walls of the dish. The mycelium is white and felty. *Sclerotinia nivalis* has small sclerotia up to 7 mm in diameter, spherical. In terms of colony growth, the sclerotia are formed in concentric circles. In the center of the colony, the mycelium acquires an olive-gray hue. *Botrytis cinerea* on the plant can manifest both as an olive-gray sporulation and as a grayish, cotton-like mycelium. The sclerotia are under 2 cm in length, black, irregular in shape, flat in cross-section, grayish, and may appear in the upper layers of root crop tissues. On PGA, the colonies are light gray. The sclerotia are small, black,

often flat and irregularly shaped, unevenly distributed over the surface of the colony. A comparison of pathogen cultures is shown in Figure 2.

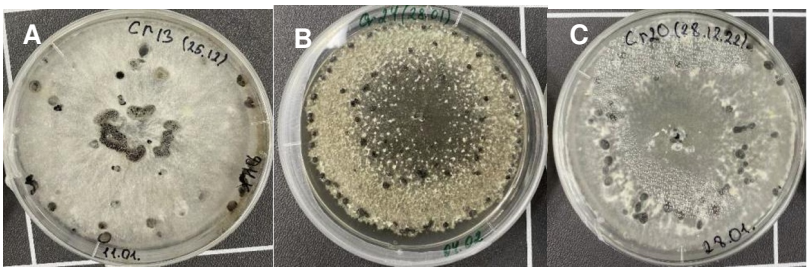

Fig. 2. Appearance of colonies of pathogens. **A**: *Botrytis cinerea*, **B**: *Sclerotinia nivalis*, **C**: *Sclerotinia sclerotiorum*.

S. sclerotiorum - a long-known pathogen of carrots, widespread in our territory. Most of the samples with the identification of this pathogen fall in the Samara region. The proportion of these samples was 6.9%. But the pathogen was also detected in Novgorod (1.4%), Sverdlovsk (1.4%), Bryansk (1.4%), Tver (2.8%) and Moscow (4.2%) regions. In total, *S. sclerotiorum* was registered in 13 samples. It was isolated from all regions of the Russian Federation, except the Stavropol Territory. The less common is *S. nivalis* [5]. There is only 7 samples, where pathogen was identified: in Samara Region (1.4%), Republic of Tatarstan (5.6%), Nizhny Novgorod Region (1.4%), Moscow Region (1.4%). More samples *S. nivalis* was identify in Samara Region and from Republic of Tatarstan. But in general, it can be concluded that the distribution of this species in the territory of the Russian Federation is limited.

Part of the study was devoted to exploring the possibility of expanding the scope of Miravis ®, SC (registered for carrots against alternariosis and powdery mildew) and Cideli Top ™, SL (registered against alternariosis and powdery mildew).

Discs of 2 mm isolate culture were selected as a source of infection for inoculation *S. sclerotiorum* from Samara Region. The results of the study of treatment with Cideli Top ™, SL on discs and carrot leaves in points are presented in Table 1.

Table 1. The results of evaluating the effectiveness of the use of Miravis ®, SC and Cideli Top ™, SL on carrot leaves and disks of carrot.

Fungicide's name	**Concentration, %**	**Score of damage of leaves/rot crop after inoculation, points**	
		3rd day	**6th day**
Miravis ®, SC	Control	2/2	4/3
	0.18	1/0	1/0
	0.25	0/0	0/0
	0.30	0/0	0/0
	0.33	0/0	0/0
			R, % 6.3 / 0
			B.E.,% 93.8 / 100
Cideli Top ™, SL	Control	2/2	4/3
	0.19	2/2	3/3
	0.25	1/2	3/3
	0.30	1/1	1/1
	0.33	0/1	0/1
			R, % 43.8 / 50.0
			B.E.,% 56.3 / 33.3

In the experiments, on plant fragments treated with Miravis ®, SC (adepidyn 200 g/l), the manifestation of symptoms was practically not observed. Carrot root fragments-maintained turgor, tissues were not soft, and necrosis was not observed. The leaves lost their green hue slightly over 3 days due to a lack of nutrients. Slight necrosis was found only at the lowest concentration of the product. No signs of disease were observed in the other variants.

On plants treated with Cideli Top ™, SL (diphenoconazole 125 g/l + cyflufenamide 15 g/l), the disease developed more slowly. In all variants, except the highest concentration, tissue death began to some extent on the carrot cross-sections. Similar processes occurred on the leaves. At the 0.19% treatment, the pathogen's mycelium was manifested.

As a result of the experiment, the most effective fungicide against white rot on carrot - Miravis ®, SC (adepidyn 200 g/l) which stops the development of the disease at a minimum concentration. The biological efficiency on carrot fragments was 100%.

Cideli Top ™, SL has shown the ability to restrain the development of the disease only at the highest concentration of the solution. Its biological efficiency on carrot discs was 33.3%.

It can be assumed that Miravis® treatment during the growing season can reduce the risk of developing white rot in storage conditions. Therefore, it is recommended to test this fungicide in the field against the white rot of table carrots.

4 Conclusion

As a result of laboratory studies, it was found that the most common pathogens of table carrot root crops are fungi of genus *Penicillium, Fusarium, Mucor, Aspergillus, Pythium, Alternaria, Botrytis* and *Sclerotinia.* The most harmful pathogens of rot during storage are *S. sclerotiorum* and *S. nivalis,* causes white rot of carrot. These pathogens cause tissue maceration, which leads to the death of a large part of the plant tissues. In three days, white rot affects more than 50% of the tissues on carrot discs.

Against this disease, the maximum indicators of biological efficacy (100%) with artificial infection of root crops were noted of using Miravis®, SC in concentrations from 0.18 to 0.33%, and it can be recommended for use during the growing season to contain white rot during storage.

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